THE INTRASPECIFIC VARIATION OF PRATYLENCHUS BRACHYURUS

By

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For Julie
My Adorable Wife

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Two differentiating techniques (host response and isoelectric focusing electrophoresis) were used to study intraspecific variation among five Pratylenchus brachyurus populations selected from different geographical regions and hosts. Although small morphological differences were observed among the populations, each population was identifiable as P. brachyurus. No nematode population had an effect ($P \leq 0.05$) on fresh shoot or root weights of plants. Each population reproduced similarly on each of the host plants. Analysis of enzymes from 250 females by isoelectric focusing electrophoresis in conjunction with enzyme-staining systems revealed differences in protein banding patterns among populations. Three distinct phenotypic groups were observed in the malate dehydrogenase and phosphoglucomutase systems. The number of bands among populations varied from

two to eight in the malate dehydrogenase system, and from three to five in the phosphoglucomutase system. Only one phenotype, composed of one band, was observed for all populations in the phosphoglucose isomerase system.

CHAPTER 1
INTRODUCTION

Plant-parasitic nematodes are microscopic worms that damage most agricultural crops. Estimated overall average annual yield loss of the world's major crops due to damage by plant-parasitic nematodes is 12.3% (70). Monetary losses due to nematodes on 21 crops, 15 of which are "life sustaining", were estimated in 1987 at $77 billion annually. The United States' portion of these losses was $5.8 billion (70).

Nematode management is a practice whereby plant-parasitic nematodes are maintained at levels that do not cause economic losses (32). Nematode management practices generally include the application of fumigant and nonfumigant nematicides, the use of cultural practices, and resistant cultivars. The number of currently registered nematicides is small and inadequate to meet the many needs of growers. Several of these nematicides may be associated with groundwater pollution or present a potential risk to animal life (46). Crop rotation is one of the most important cultural methods for managing nematodes (45), but its usefulness is limited because before it can be properly implemented the target nematode must be correctly identified

and its host range known. Similar constraints applied to the successful use of plant resistance to nematodes.

Species of the genus Pratylenchus can be particularly difficult to identify. Several species are morphologically similar and the intraspecific variation can be great (52). Many species are erected on weak, inadequate comparisons, subjective criteria, a minimal number of specimens, and (or) insufficient diagnostic data (28). Not all species are accepted as being valid by some nematologists (28). An understanding of the intraspecific variability among and within species of this genus is essential in developing proper nematode management programs.

Pratylenchus brachyurus (Godfrey, 1929) Filipjev & Stekhoven is a migratory endoparasite of important agricultural crops. The nematode forms tunnels and cavities as it feeds and moves through roots. It causes extensive damage to the cortex and in some cases to the vascular system of the plant (51). Numerous pathogenic studies were conducted on the nematode in a variety of economically important crops (6, 9, 17, 18, 29, 30, 48, 51, 62, 71, 76, 79, 87).

Pratylenchus brachyurus is widely disseminated throughout the warmer regions of the world (9, 10). The nematode is also widely distributed throughout the southern portion of the United States (77). It is the most ubiquitous species of Pratylenchus in Florida, and it is associated

with plant roots in 54 of 67 counties in the state (Division of Plant Industry, unpubl.). In Florida, Pratylenchus spp. were detected in 93% of all citrus groves and P. brachyurus was identified in 90% of them (83).

Records of intraspecific variability within P. brachyurus are limited to morphological studies (68, 82). Field observations, however, suggested behavioral differences within P. brachyurus populations (D. W. Dickson and R. A. Dunn, personal communication). The presence of P. brachyurus biotypes has not been documented, but their existence is suggested by variation in numbers of P. brachyurus extracted from roots of Citrus limon (L.) Burm. f. seedlings when inoculated with different nematode isolates (55).

Races within species of plant-parasitic nematodes are recognized as morphologically similar, but with different host preferences (81). Other terms, such as biotype and pathotype, are also used to describe such populations (15, 74). These physiological races are documented in several species of plant-parasitic nematodes (15). Differentiation of races is usually determined with differential host tests, as for example with Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949, races 1-4 (31). Separation of races can be a complicated task, such as is the case for Heterodera glycines Ichinohe, 1952 (66). Newer and better

methods are needed to separate races quickly and reliably in order to design more successful nematode management programs.

Biochemical approaches in nematode taxonomy have considerable potential for assisting in the identification and characterization of nematodes as well as establishing phylogenetic relationships (39). The most common biochemical methods include the analyses of proteins and nucleic acids. Currently, restriction fragment analyses, DNA-RNA hybridization, and nucleotide sequencing are being used widely for systematic purposes (3). Phenotypic variation among certain populations of H. glycines was detected using isoelectric focusing electrophoresis in conjunction with enzyme-staining systems (60). Most biochemical data are obtained from mass homogenates of nematodes since individual nematodes contain small quantities of protein. However, techniques such as isoelectric focusing electrophoresis are sensitive enough to detect proteins from a single female of H. glycines (34, 64).

The objective of this research was to determine whether intraspecific variability existed among five P. brachyurus populations collected from diverse geographical regions and hosts based on their response on seven crop plant species and enzyme profiles obtained by isoelectric focusing electrophoresis.

CHAPTER 2
IDENTIFICATION, ANATOMY, AND MORPHOMETRICS OF PRATYLENCHUS BRACHYURUS

Introduction

Pratylenchus brachyurus, a migratory endoparasitic nematode, was first described by Godfrey as the primary cause of pineapple-root disease in Hawaii (29). The disease is characterized by brown root lesions and in more severe cases by the death of many of the branch rootlets and some main roots. Godfrey named the nematode Tylenchus brachyurus and placed it in the subgenus Chitinotylenchus (29). He made reference to other nematodes of similar general appearance: T. musicola Cobb, T. pratensis de Man (=T. penetrans Cobb), T. coffeae Zimmermann, and T. sacchari Soltwedel, all of which were later also placed in the genus Pratylenchus. Godfrey noted that one major difference between P. brachyurus and other Pratylenchus spp. was the absence of males in P. brachyurus (29).

The genus Pratylenchus was first proposed by Filipjev in 1934 with Tylenchus pratensis de Man, 1880 as the type-species (24). According to the International Code of Zoological Nomenclature, a new genus name must be accompanied by a statement that purports to give characters

differentiating the new genus plus the name of the type-species (80). Two years later when Filipjev characterized the genus, the new name was universally accepted (25). Since the establishment of the genus, 89 species have been proposed (28). But not all of them were accepted by most nematologists (28). Initially, the taxonomy of the genus was confused chiefly because the identity of the type species, P. pratensis (de Man), was not unambiguously established (52).

Since Godfrey's description, the following species were synonymized as P. brachyurus: Tylenchus brachyurus Godfrey, 1929; T. (Chitinotylenchus) brachyurus Godfrey, 1929 (Filipjev, 1934); Anguillulina brachyura (Godfrey, 1929) Goodey, 1932; A. brachyura (Godfrey, 1929) Goodey, 1932 (Scheider, 1939); P. pratensis Thorne, 1940; P. leiocephalus Steiner, 1949; and P. steineri Lordello, Zamith, and Book, 1954 (10).

Several reviews of the genus Pratylenchus were published (52, 53, 73). The latest compendium reviewed 89 described species, but only 49 of them were considered valid (28). Only a few investigators have reported studies on the extent of variation within certain species. The degree of intraspecific variation must be known if we are to understand the morphology of existing species and to avoid unnecessary creation of new ones (28).

Identification of Pratylenchus species is based primarily on mature females. Several species are morphologically similar, and intraspecific variation can be great. Therefore nematode taxonomic expertise is required when working with this genus (52, 73).

The following characters are considered to have diagnostic value: number of annules and shape of lip region, length of stylet, annulation and shape of tail tip, position of vulva, length of posterior uterine branch, body length, presence and shape of the spermatheca, number of lines in the lateral field, and presence or absence of males (52, 53). Various degrees of intraspecific variability are observed in several surface features of the nematode through scanning electron microscope studies (11).

Cultures of Pratylenchus brachyurus and P. coffeae (Zimmermann) Filipjev & Stekhoven from individual females revealed that the shape of the lip region had variable lip shapes within and between the two species (82). Intraspecific variation is observed in the number of lip annules in P. brachyurus, P. coffeae, P. scribneri Steiner, P. vulnus Allen & Jensen, P. zeae Graham, but not in P. penetrans (Cobb) Filipjev & Stekhoven (68, 84). Electron microscope studies of the lip region of Pratylenchus spp. clearly show the existence of variation among and within species of the genus (11).

The length of the stylet is one of the most reliable characters for identifying Pratylenchus spp. (2, 27, 75, 86). Although the stylet length is one of the least variable characters, the shape of the stylet knobs varies among some species (68, 84).

In the original description of P. brachyurus, variations of tail shapes were reported (29). Intraspecific variation in the shape and length of the tails of P. brachyurus, P. coffeae, P. hexincisus Taylor & Jenkins, P. penetrans, and P. zeae was demonstrated using natural and single gravid female cultures (82, 84, 86). Electron and light microscope studies on the genus Pratylenchus reveal that the number of annules in the tail vary within and among species (11, 68, 82, 84). Most species have a mean number of 18 to 24 annules with overlapping ranges; therefore, this character is of little use in differentiating species (11). The lateral field in Pratylenchus spp. has four mutually equidistant straight lines (68). Although errors in interpretation and, to some extent, the intraspecific variation in the lateral field are too great to allow this to be a good diagnostic character, it is a major character used in separating some species, such as P. hexincisus. (11, 68, 84).

Most species descriptions of Pratylenchus include values for ratios of "a" (total body length / body width), "b" (total body length / distance from anterior end to

posterior end of esophagus), and "c" (total body length / tail length) (78), plus the distance of the vulva from the anterior end as a percent of the body length ("V"). The first three ratios are highly variable, whereas the "V" value is not (68, 75). The "V" value is one of the least variable characters in the genus, and it is regarded as a good feature in species differentiation (2, 27, 52, 53, 68, 73, 86).

Intraspecific variability may be due, at least in part, to the effect of the environment on the nematode. Host nutrition, crowding, and temperature influenced nematode size in P. brachyurus and P. zeae (56, 57).

Pratylenchus brachyurus bears the typical characteristics of the genus. It is recognized by the angular margins of the lip region, which is set off from the body and bears two distinct annules. The spermatheca is almost invariably empty. The vulva is located near the posterior end. The tail shape is subcylindrical with smooth, rounded, or truncated tail terminus, with no striations around it. The female dimensions are body length = 0.39-0.75 mm, a = 15-29, b = 5-10, c = 13-18, V = 82-89%, stylet = 17-22 μm, egg = 70-80 X 20-28 μm. Males are extremely rare, but general appearance is similar to the females. They have a single outstretched testis. The male dimensions are body length = 0.46-0.56 mm, a = 27-29, b = 6, c = 21, T = 51-53%, stylet = 19 μm (52, 73).

In this chapter the objective was to provide morphometric data on the identification of five Pratylenchus brachyurus populations selected from different geographical locations and hosts, and to determine the extent of intraspecific morphological variation within each population.

Materials and Methods

The origins and sources of the different Pratylenchus brachyurus populations are 101 from corn (Zea mays L. cv. Pioneer 304C) in Alachua County, Florida; 102 from peanut (Arachis hypogaea L. cv. Florunner) in Alachua County, Florida; 103 from peanut cultivar Florunner in Tift County, Georgia; 105 from soybean (Glycine max (L.) Merr. cv. Forrest) in North Carolina; and 108 from citrus (Citrus sp.) in Polk County, Florida.

Each population was established by inoculating snap bean (Phaseolus vulgaris L. cv. Harvester) seedlings with 200 nematodes (mixed life stages). The inoculated seedlings were maintained in a greenhouse at 25 C. Nematodes were extracted using a modified Baermann funnel (17). The infected roots were washed and cut into pieces about 1 cm long, and blended with 150 ml water for 30 seconds. The sample was washed from the blender onto a 38-μm-pore sieve, rinsed with tap water, and washed off onto a nylon screen with 1.5-mm-openings that stretched over a 11.5-cm-diameter

PVC pipe. The nylon screen was covered with Scotties tissue paper (Scott Paper Company, Philadelphia, Pennsylvania) to retain the blended roots, then placed in a plastic sandwich box (13 X 13 X 4 cm). Finally, the box was filled with tap water to cover the nylon screen forming a thin film of water around the root pieces. The nylon screen plus the tissue retained the roots and allowed the nematodes to migrate into the water contained in the box. After 72 hours of incubation, the nematodes were collected by sieving the box contents through a 38-μm-pore sieve (Figure 2-1).

Fifteen females from each nematode population were measured using a microscope fitted with a drawing tube. Measurements from live females were made within 30 minutes after mounting the specimens. Females that had the appearance of being well-fed were randomly picked and placed in a small drop of water on a microscope slide. A ring of Zut was placed around the drop with a small painting brush, and a coverslip was gently lowered onto the ring. The excess water and air were slowly forced out by gently pressing the coverslip with a dissecting needle. Five minutes later the nematodes were measured as they lay motionless.

Specimens were measured with the use of a drawing tube. A line was drawn through the middle of the female body from the head to the tail at 20X magnification. The excretory pore, the vulva, and the anus measurements were taken from the center of each orifice and a mark was placed onto the

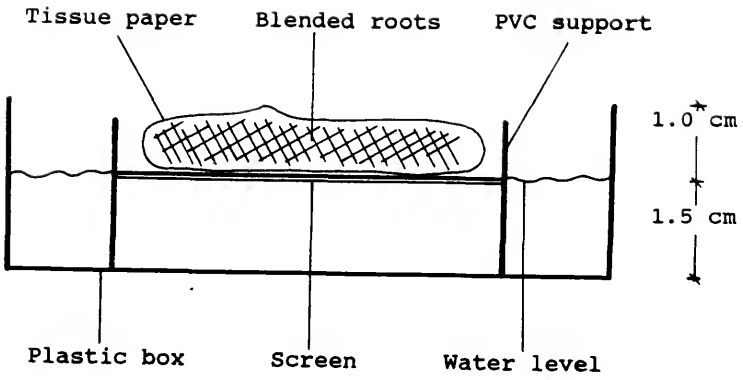


Figure 2-1. A cross section of the modified Baermann funnel used for nematode extraction. The screen was glued between two sections of PVC pipe used for support. Inside diameter of PVC pipe = 10.2 cm (drawing not to scale).

line drawn for the body length. The stylet length, the width of the nematode, the shape of the lip region, and the shape of the tail were drawn at 100X magnification using an oil immersion objective. The nematode line was measured with a map reader and redrawn on a separate straight line which subsequently was measured with a ruler and converted into micrometers. The length of the stylet and the width of the nematode were taken directly from the drawings.

The following measurements, ratios, and characters were recorded: body length, body width, stylet length, distance from excretory pore to anterior body end as % of total body length, distance from vulva to anterior body end as % of total body length, "a" and "c" ratios, and the number of lines in the lateral field. The ratios "a" (total body length / body width) and "c" (total body length / tail length) were calculated using de Man indices (78). The extent of variability in the characters was expressed by calculating the mean, range, standard deviation, and coefficient of variation.

Results

All measurements obtained from all specimens were within the reported ranges for P. brachyurus (52). There were no significant morphological differences among any of the populations studied. It is concluded that all of the populations were P. brachyurus, and that they do not differ

morphologically from each other (Tables 2-1 to 2-5). All of the specimens had the typical truncated head composed of two lip annules, three large amalgamated stylet knobs, and four lines in the lateral field.

The shape of the amalgamated stylet knobs was rounded, but one-third of the specimens of the peanut population from Florida had a cup-shaped anteriorly directed ventral knob (Figure 2-2). The shape of the tail tip was smooth and bluntly rounded, but it also varied from flat in two specimens to indented in two others (Figure 2-3).

Among the measurements obtained, the stylet length and the position of the vulva as a percent of the total body length were the least variable characters and thus the most reliable ones in the identification of the species. The stylet length varied from 17 to 20 μm with a mean of 18.4 μm, and a coefficient of variability (CV) of 3.0% among all populations. The position of the vulva varied from 80 to 88.2% with a mean of 85% and a CV of 1.7% among all populations.

All the other characters measured, such as body length, body width, tail length, vulva-anus distance, "a" and "c" ratios, and the excretory pore as a percent of total body length had relatively high coefficients of variability across all populations.

Table 2-1: Measurements of mature females (n=15) of Pratylenchus brachyurus 101 originating from 'Pioneer 304C' corn, Alachua County, Florida.

Morphological characters	Range	Mean	SD[a]	CV(%)[b]
Measurements in μm:				
Body length	521.9-687.5	585.6	48.9	8.4
Body width	20.0-30.6	24.9	3.2	12.9
Stylet length	17.1-18.8	18.0	0.6	3.0
Tail length	25.0-40.6	30.4	3.7	12.2
Vulva-anus distance	43.8-70.3	56.0	7.2	12.9
Measurements as %:				
Position of the excretory pore as % of total body length (n=11)	9.2-16.8	14.4	2.1	14.6
Position of the vulva as % of total body length	83.2-87.7	85.2	1.4	1.6
Ratios:				
a	20.4-27.3	23.8	2.4	10.0
c	16.7-22.9	19.4	2.0	10.3

[a]SD = Standard deviation.
[b]CV = Coefficient of variation.

Table 2-2: Measurements of mature females (n=15) of Pratylenchus brachyurus 102 originating from 'Florunner' peanut, Alachua County, Florida.

Morphological characters	Range	Mean	SD[a]	CV(%)[b]
Measurements in μm:				
Body length	553.1-712.5	646.7	47.7	7.4
Body width	26.5-35.3	30.9	2.3	7.4
Stylet length	17.0-20.0	18.5	0.8	4.3
Tail length	25.0-37.5	30.8	3.5	11.4
Vulva-anus distance	46.9-100.0	70.2	13.7	19.5
Measurements as %:				
Position of the excretory pore as % of total body length (n=14)	14.9-18.5	16.2	0.9	5.5
Position of the vulva as % of total body length	80.0-87.6	84.4	2.0	2.4
Ratios:				
a	19.1-22.9	20.9	1.2	5.7
c	17.7-27.2	21.2	2.6	12.3

[a]SD = Standard deviation.
[b]CV = Coefficient of variation.

Table 2-3: Measurements of mature females (n=15) of Pratylenchus brachyurus 103 originating from 'Florunner' peanut, Tift County, Georgia.

Morphological characters	Range	Mean	SD[a]	CV(%)[b]
Measurements in μm:				
Body length	606.2-712.5	657.4	28.4	4.3
Body width	28.2-32.9	30.4	1.5	4.9
Stylet length	17.6-19.4	18.7	0.6	3.2
Tail length	31.3-37.5	33.5	2.4	7.2
Vulva-anus distance	46.9-75.0	64.9	7.1	10.9
Measurements as %:				
Position of the excretory pore as % of total body length (n=11)	13.9-17.7	15.6	1.0	6.4
Position of the vulva as % of total body length	82.6-88.2	85.0	1.4	1.6
Ratios:				
a	19.9-23.7	21.6	1.0	4.6
c	16.9-22.8	19.7	1.6	8.1

[a]SD = Standard deviation.
[b]CV = Coefficient of variation.

Table 2-4: Measurements of mature females (n=15) of Pratylenchus brachyurus 105 originating from 'Forrest' soybean, North Carolina.

Morphological characters	Range	Mean	SD[a]	CV(%)[b]
Measurements in μm:				
Body length	521.9-687.5	618.8	44.8	7.2
Body width	23.5-31.2	27.6	2.4	8.7
Stylet length	17.0-20.0	18.5	0.7	3.8
Tail length	28.1-40.6	32.9	3.1	9.4
Vulva-anus distance	53.1-68.8	61.0	4.7	7.7
Measurements as %:				
Position of the excretory pore as % of total body length (n=10)	13.4-15.9	14.9	0.9	6.0
Position of the vulva as % of total body length	82.0-87.3	84.7	1.5	1.8
Ratios:				
a	20.3-26.0	22.5	1.5	6.7
c	15.8-22.3	18.9	1.8	9.5

[a]SD = Standard deviation.
[b]CV = Coefficient of variation.

Table 2-5: Measurements of mature females (n=15) of Pratylenchus brachyurus 108 originating from citrus, Polk County, Florida.

Morphological characters	Range	Mean	SD[a]	CV(%)[b]
Measurements in μm:				
Body length	515.6-659.4	583.5	42.1	7.2
Body width	20.6-25.3	22.5	1.4	6.2
Stylet length	18.2-18.8	18.5	0.1	0.5
Tail length	25.0-37.5	31.9	2.9	9.1
Vulva-anus distance	37.5-59.4	49.2	4.8	9.8
Measurements as %:				
Position of the excretory pore as % of total body length (n=14)	9.2-16.8	14.4	2.1	5.5
Position of the vulva as % of total body length	84.7-87.9	86.1	0.9	1.0
Ratios:				
a	21.9-29.3	26.8	2.1	8.0
c	15.0-24.9	18.5	2.5	13.5

[a]SD = Standard deviation.
[b]CV = Coefficient of variation.

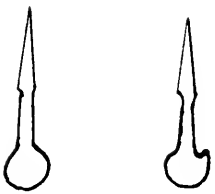


Figure 2-2. Stylet knob shapes of Pratylenchus brachyurus: a = rounded amalgamated knobs which are the typical shape for the species, b = cup-shaped, anteriorly directed knob.

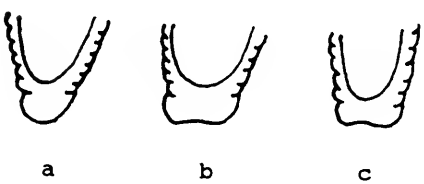


Figure 2-3. Tail tip shapes of Pratylenchus brachyurus: a = bluntly rounded (typical shape for the species), b = flat, c = slightly indented.

Discussion

Morphological studies confirmed the identity of the five nematode populations as P. brachyurus. Although slight morphological variations were observed among populations, they could not be separated morphologically.

The lip region of all specimens was similar to that described for P. brachyurus (68, 73, 82). Variations reported include rounded lips and the presence of two annules on one side and three on the other side. There was no apparent variation in shape or number of lip annules in specimens from each population used in this study.

Stylet length was reported as one character with limited variation in P. brachyurus (68, 82). In one study, stylet length of 50 specimens ranged from 17.4-19.2 μm with a mean of 18.4 μm and a CV of 2.6% (68). In another case, the range of 216 specimens was 17-19 μm with a mean of 17.9 μm and a CV of 2.4% (82). In this study, combined data from all nematode populations showed a range of 17-20 μm with a mean of 18.4 μm and a CV of 3.0%. The variation in stylet knob shapes observed was similar to those reported previously for P. penetrans and P. zeae (68, 84).

Vulva position was the morphological character with the least variation. In previous studies vulva position of P. brachyurus ranged from 80-87% with a mean of 85% and a CV of 1.7% (68). Combined data from all nematode populations studied showed a range of 80-88.2% with a mean of 85% and a CV of 1.7%.

Most of the specimens observed in this study had smooth, bluntly rounded tails with a hyaline portion at the terminus as described previously for the species (68). The tail length was more variable than the shape. In one study, tail length of P. brachyurus ranged from 20-40 μm with a mean of 29.3 μm and a CV of 11% (82). In another study it ranged from 25.2-37.8 μm with a mean of 31.5 μm and a CV of 7.4% (68). Combined data from all populations studied here showed a range of 25.0-40.6 μm with a mean of 31.9 μm and a CV of 9.8%.

The distance between the vulva and the anus is a good diagnostic character to differentiate between P. coffeae and P. brachyurus (82). The distance between the vulva and the anus in P. brachyurus ranges from 42-75 μm with a mean of 56.5 μm and a CV of 10.9% (82). Another study reports a range of 39-64.2 μm with a mean of 50.6 μm and a CV of 10% (68). Similar results were obtained in this study. The combined data from all populations showed a range of 37.5-100 μm with a mean of 60.3 μm and a CV of 12.2%.

The degree of intraspecific morphological variability among P. brachyurus populations depended on the morphological characters studied. Morphometric data is necessary in the identification of Pratylenchus spp. but it should be used with caution. In addition to considering the normal intraspecific variation that can occur, care must be exercised in obtaining measurements. Differences between

measurements were obtained when the same specimen was measured by several experienced researchers, and even when one specimen was measured by the same researcher on different days (27).

CHAPTER 3
HOST SPECIFICITY OF FOUR PRATYLENCHUS BRACHYURUS POPULATIONS

Introduction

Pratylenchus brachyurus causes damage to many tropical and subtropical crops (29, 76). This nematode is widely distributed throughout the tropics and it is found in association with many plant species (see Chapter 1). Taxonomic separation of the species of Pratylenchus is difficult because they exhibit little morphological diversity. Difficulties often arise from underestimation of intraspecific variability of certain morphological characters currently used for distinguishing species (see Chapter 2, 68). The validity of some species is questionable (28).

Populations of plant-parasitic nematodes that are designated as biological or physiological races are recognized as morphologically similar, but with different host preferences (81). Physiological races are documented in certain species of plant-parasitic nematodes, such as those from the genus Meloidogyne (85), but the presence or absence of races within P. brachyurus has not been documented. The presence of P. brachyurus races is suggested by variation in

numbers of P. brachyurus extracted from roots of Citrus limon (L.) Burm. f. seedlings when inoculated with different nematode populations (55). Field observations suggest possible behavioral differences within P. brachyurus populations (D. W. Dickson and R. A. Dunn, personal communication). The morphometrics of P. brachyurus vary considerably in response to geographical locations, unfavorable hosts, overcrowding, and high temperatures (56, 57). Intraspecific morphological variation is demonstrated using populations that originated from single gravid females (82, 84).

In this chapter the objectives were to determine the usefulness of different host plants in separating four P. brachyurus populations obtained from different geographical locations and to study host response to each population.

Materials and Methods

The designation and sources of the four populations of lesion nematode, P. brachyurus, are given in Chapter 2. Seven plant species, representing a wide diversity of plant types and reported as hosts for P. brachyurus, were evaluated in a greenhouse. The differential plants were alfalfa (Medicago sativa L. cv. Florida 77); snap bean (Phaseolus vulgaris L. cv. Harvester); citrus cultivar Rough Lemon; corn cultivar Pioneer 304C; peanut cultivar

Florunner; soybean cultivar Braxton; and tomato (Lycopersicon esculentum Mill. cv. Rutgers).

Nematode inoculum was extracted as described in Chapter 2. Germinated seedlings of the different plant types with 3-cm-long radicles were transplanted into 900 cm^3 of steam-pasteurized sandy loam soil in individual 15-cm-diameter clay pots. When the seedlings were 1 week old, the soil was infested separately with 300 nematodes (mixed life stages) per pot (approximately 0.33 nematodes/cm^3 soil). The nematodes in an aqueous suspension were poured into two small holes, each 5-cm deep, near the base of the seedling.

The experiment was conducted in the summer of 1986 with six replications and repeated in the fall of 1987 with five replications. Plants were harvested 64 days after inoculation and shoot fresh weight, root fresh weight, and the nematode density levels were determined. Densities of nematodes in the soil were determined by processing 100 cm^3 of soil by the sugar-flotation-centrifugation method (44). Root densities were determined by processing 10 g of roots by the method described in Chapter 2.

A factorial experiment was conducted using a split-plot design. The plants were blocked by replicate using plant type as a main plot and populations as a subplot on a greenhouse bench. The greenhouse temperature was maintained at 25 $\pm$ 5 C. The effects by the plant, by the population, and interactions of the two were determined by analysis of variance.

Results

No nematode population affected fresh shoot or root weight when compared to the uninoculated controls in either experiment (Tables 3-1 and 3-2). Final population densities were different ($P \leq 0.05$) on 'Harvester' snap bean in the two experiments and on 'Braxton' soybean in the second experiment (Tables 3-3 and 3-4). In the first experiment, populations 102 and 103, both from peanut, had higher final population densities on 'Harvester' snap bean than the other two populations ($P \leq 0.05$); however, there were no differences in population densities between the two populations from peanut or between the other two populations, which originated from soybean or corn. These observations were the same whether the root or the soil nematode population densities were compared (Tables 3-3 and 3-4). In the second test, population 101 from Florida had higher population density in 'Harvester' snap bean and in 'Braxton' soybean than the other three populations ($P \leq 0.05$). There were no differences in population densities among the other three populations in either of the two hosts (Tables 3-3 and 3-4). Nematodes were also extracted from 100 cm^3 of soil in 1987; however, since the numbers were very low (5/100 cm^3 of soil) no data is shown here.

Table 3-1: Shoot weights (g) of seven plant species 64 days after inoculation with four Pratylenchus brachyurus populations and the uninoculated control.

Plant species	Year	P. brachyurus population[a]				
		101	102	103	105	Control
Alfalfa	1986	34[b]	33	33	30	39
	1987	49	47	43	46	50
Snap bean	1986	55	47	45	50	60
	1987	64	91	70	80	65
Citrus	1986	46	51	56	50	43
	1987	10	9	9	11	10
Corn	1987	172	188	177	169	182
	1986	144	128	117	176	149
Peanut	1986	69	74	70	69	67
	1987	63	63	57	53	61
Soybean	1986	101	93	93	110	99
	1987	23	27	28	22	26
Tomato	1986	132	125	131	129	131
	1987	101	87	112	102	100

[a]Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina.

[b]Data are the means of six (1986) and five (1987) replications. No significant ($P \leq 0.05$) differences were found among means in any row, according to least significant difference test.

Table 3-2: Root weights (g) of seven plant species 64 days after inoculation with four Pratylenchus brachyurus populations and the uninoculated control.

Plant species	Year	P. brachyurus population[a]				
		101	102	103	105	Control
Alfalfa	1986	35[b]	28	27	21	36
	1987	43	35	39	42	40
Snap bean	1986	20	18	13	21	19
	1987	36	31	34	42	37
Citrus	1986	34	48	49	46	42
	1987	5	4	5	5	4
Corn	1987	171	149	139	135	142
	1986	77	60	66	84	72
Peanut	1986	24	18	17	19	15
	1987	18	21	17	17	21
Soybean	1986	82	62	56	61	60
	1987	20	26	26	17	22
Tomato	1986	32	26	29	26	28
	1987	46	49	59	42	40

[a]Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner peanut', Tift County, Georgia; 105- 'Forrest' soybean, North Carolina.

[b]Data are the means of six (1986) and five (1987) replications. No significant differences ($P \leq 0.05$) were found among means in any row, according to least significant difference test.

Table 3-3: Final population densities (nematodes per g of root) of four Pratylenchus brachyurus populations 64 days after inoculation.

Plant species	Year	P. brachyurus population[a]			
		101	102	103	105
Alfalfa	1986	21[b] a	48 a	29 a	14 a
	1987	1 a	3 a	1 a	0 a
Snap bean	1986	158 b	253 a	266 a	125b
	1987	22 a	9 b	9 b	4 b
Citrus	1986	0 a	0 a	0 a	0 a
	1987	1 a	1 a	0 a	0 a
Corn	1986	23 a	25 a	30 a	18 a
	1987	12 a	5 a	4 a	4 a
Peanut	1986	6 a	9 a	16 a	7 a
	1987	6 a	4 a	5 a	3 a
Soybean	1986	23 a	29 a	30 a	11 a
	1987	23 a	8 b	9 b	7 b
Tomato	1986	89 a	41 a	44 a	36 a
	1987	19 a	2 a	2 a	2 a

[a]Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina.

[b]Data are the means of six (1986) and five (1987) replications. Means within rows followed by the same letter are not different ($P \leq 0.05$) according to least significant difference test.

Table 3-4: Final population densities (nematodes per 100 cm^3 of soil) of four Pratylenchus brachyurus populations 64 days after inoculation.

Plant species	Year	P. brachyurus population[a]			
		101	102	103	105
Alfalfa	1986	24[b] a	32 a	16 a	8 a
Snap bean	1986	37 b	192 a	200 a	42 b
Citrus	1986	0 a	0 a	0 a	0 a
Corn	1986	139 a	82 a	84 a	96 a
Peanut	1986	1 a	2 a	1 a	1 a
Soybean	1986	5 a	5 a	7 a	2 a
Tomato	1986	138 a	73 a	71 a	41 a

[a]Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina.

[b]Data are the means of six replications. Means within rows followed by the same letter are not different ($P \leq 0.05$) according to least significant difference test.

None of the nematode populations were able to reproduce on citrus in either of the two experiments before the experiment was terminated. Data from the final population densities of the four P. brachyurus populations on selected hosts are given in the Appendix.

Discussion

This attempt to separate populations of P. brachyurus failed to discern behavioral differences on seven species of crop plants. The patterns observed in the final population densities in the two experiments were not consistent, suggesting that none of the populations studied were different from each other. The smaller numbers obtained in the second experiment were due, in part, to the time of the year it was conducted.

Pratylenchus brachyurus is pathogenic to at least one type of citrus, Citrus aurantium, 'Sour Orange' (6), and can be found associated with 'Rough Lemon' roots (82). Although no pathogenicity was observed on citrus here, the lack of reproduction of the nematode in citrus cultivar 'Rough Lemon' under the conditions of the two experiments indicates a possible nonhost relationship. Other factors that might be involved include unsuitability of greenhouse cultural conditions and insufficient time for nematode development.

Data from these tests provide no support for postulating the presence of races within P. brachyurus, but

the possibility cannot be excluded either until citrus populations and other populations are tested with additional host plants.

CHAPTER 4
COMPARISON OF ENZYME PHENOTYPES OF FIVE PRATYLENCHUS BRACHYURUS POPULATIONS

Introduction

The biochemical approach to nematode taxonomy has considerable potential for assisting in the identification and characterization of these organisms as well as establishing phylogenetic relationships (39). The application of comparative biochemistry to problems of systematics has taken a variety of approaches. Serology (38, 54), analysis of lipids and fatty acids (49), analysis of proteins (13, 14, 20, 23, 35, 36, 40, 61, 88), and analysis of nucleic acids (4, 5, 12, 42, 47, 59, 60, 63) are used on nematodes.

Proteins are a manifestation of the sequence of nucleotides in a gene, and analysis of these macromolecules provides a reliable approach for comparing genotypes of organisms (39). Biochemical data can be used to differentiate sibling nematode species that are morphologically indistinguishable with routine morphological examination, but are physiologically distinct (33).

The primary problem in biochemical systematics is to find those chemical characters that will be most valuable in

providing information for taxonomy (39). Data that contribute to the characterization of species and races of nematodes are particularly valuable because of the important role of plant-parasitic nematodes in agriculture.

The protein extraction technique used, the source, and the general handling of the protein could be determining factors in the success of protein analysis. It is particularly important in the case of certain types of proteins, such as isozymes, because they are so sensitive to manipulation. Variation in protein and enzyme profiles of Meloidogyne spp. were affected by the host plant and by the growing conditions of the host (43). Protein profiles of nematodes extracted from frozen roots were different than those from nematodes extracted from unfrozen roots (13). The use of a single developmental stage is critical since differences can be obtained in profiles from different life stages (8, 13). Plant-parasitic nematodes are microscopic eelworm-shaped animals; consequently the amount of protein present in each individual is very small.

The term electrophoresis is used to describe the migration of charged particles under the influence of an electric field (1). Isoelectric focusing (IEF) can be regarded as electrophoresis within a hydrogen ion gradient (1). In IEF a stable hydrogen ion gradient that decreases progressively from anode to cathode is established by carrier ampholytes. When proteins or other amphoteric

molecules are introduced into this system, they will migrate to their corresponding surface charge in the electric field. Eventually, they will reach their isoelectric point, a zone where the net electrical charge is zero (67).

Isoelectric focusing electrophoresis in conjunction with certain enzyme-specific stain systems successfully detected intraspecific variability among populations of H. glycines and was sensitive enough to detect polymorphisms within populations (65). Analyses of mass homogenates of protein from 12 H. glycines populations using eight enzyme systems showed consistent groupings among populations, but in no case did isozyme analysis of populations correlate with the conventional race scheme based on quantitative reproduction on a set of soybean differentials (65). Four Meloidogyne spp. were distinguishable from each other by IEF of nematode egg protein profiles (50). Enzyme phenotypes from a single female soybean cyst nematode were resolved using IEF in conjunction with enzyme-specific stains (34, 64).

In this section a new technique is presented that allows the use of small numbers of vermiform nematodes for protein extraction and permits studies of a single developmental stage. This new technique was combined with ultra-thin IEF and with enzyme-stain systems in an attempt to determine similarities and differences among enzyme phenotypes from mass homogenates of females and juveniles of

five populations of P. brachyurus. The investigation reported herein is intended to add biochemical data to the morphological, ecological, and behavioral data already available for P. brachyurus.

Materials and Methods

Nematode cultures. The identification and description of the nematode populations used are given in Chapter 2. Nematodes were raised using a modified root explant technique (37). They were maintained on the corn cultivar Iochief grown in plastic petri dishes containing Gamborg's B-5 medium without auxins or cytokinins (Gibco Chem., Grand Island, New York). The cultures were incubated in the dark at 29 C. Nematodes were extracted from the culture dishes by removing a block of agar with roots and placing it in a modified Baermann funnel as described for nematode extraction from roots in Chapter 2.

Protein extraction. Two hundred and fifty females, or 450 juveniles were pipetted with the use of a controlled vacuum aspirator (26). They were placed into a depression slide coated previously with Repel-Silane (LKB, Bromma, Sweden) to produce a hydrophobic surface. The volume of water was reduced to about 20 μl by pipetting out excess water. The nematodes were stored in a 20-μl droplet of deionized water in a 25-μl capillary tube and stored in a refrigerator at 5 C. The nematodes were either used

immediately or stored overnight (Figure 4-1). The contents of the capillary tubes were placed in a 1.5- X 1.5-mm well made previously on a micro culture slide (75 X 25 mm, molded glass, with well 3 mm deep X 15 mm diameter) (Figure 4-2). The surface of the slide was coated with Repel-Silane to obtain a hydrophobic surface that prevented the spreading of the droplet, and allowed the nematodes to fall to the bottom of the well. Nematodes that did not fall were pushed into the well. The glass slide was then placed in a plastic sandwich box that contained an ice block and a mirror (Figure 4-2). The mirror, which was placed directly under the glass slide, enhanced visibility since the entire process was done under the dissecting microscope at 30X magnification. After placing the nematodes in the well, most of the water was removed with a syringe and filter paper. The nematodes, which remained attached to the walls of the well by a thin film of water, were macerated for 30 seconds with a ground glass rod made to fit the well snugly. After maceration, 2 μl of a solution containing 20% sucrose and 2% Triton X-100 (20) were added to the well and mixed thoroughly. The contents of the well were absorbed onto a 5- X 10-mm filter paper (LKB, Bromma, Sweden) and applied directly to the surface of the gel 1 cm from the anode.

Electrophoresis. The protein from 250 macerated females were subjected to isoelectric focusing in thin layer (1-mm) gels (67). The gel concentration consisted of T= 5%, C= 3%,

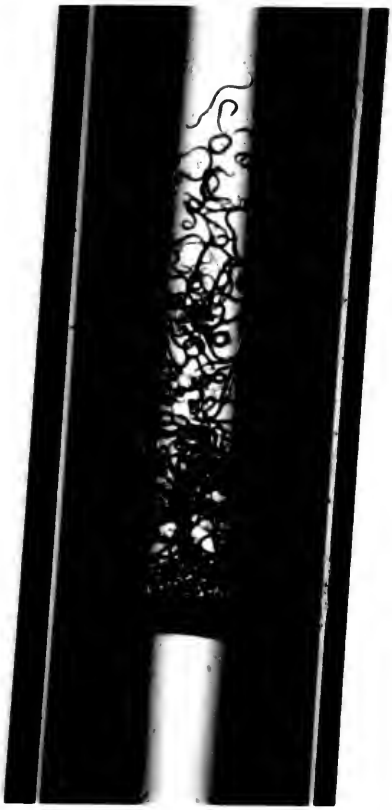
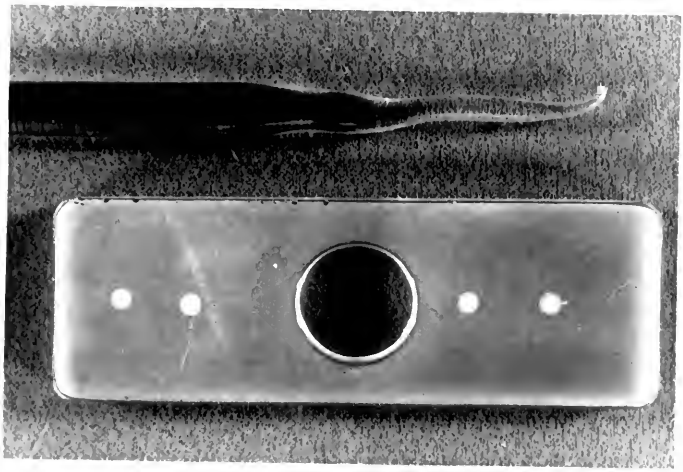
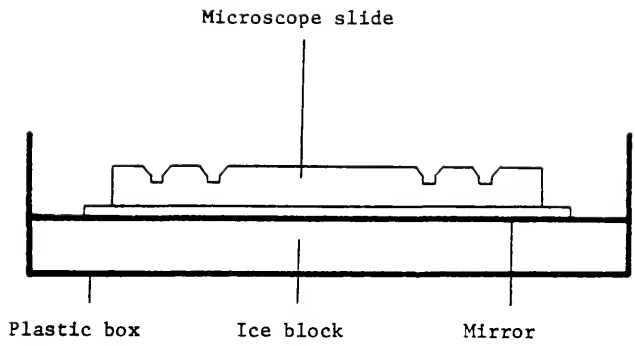


Figure 4-1. Two hundred and fifty Pratylenchus brachyurus females stored alive in a 25-μ capillary tube.

Figure 4-2. Protein extraction equipment. A) Photograph of a micro culture slide (75 X 25 mm, molded glass, with well 3 mm deep X 15 mm diameter) showing the wells and the glass rod constructed to macerate vermiform nematodes for protein extraction. B) Cross-section of the microscope slide (not to scale) used for macerating vermiform nematodes. The dimension of each well is 1.5 mm wide at the bottom, 2.5 mm wide at the surface, and 1.5 mm deep. The slide was placed over a glass mirror that lay over an ice block contained in a plastic sandwich box.



(A)



(B)

and 2.4% ampholytes with a pH range of 3.5-9.5. The LKB ultrophor system was used with 1 M H_3PO_4 in the anode and 1 M NaOH in.the cathode (67). Gels were maintained at a constant temperature of 5 C. The power was applied from a LKB macrodrive 5 constant power supply set at 1,500 volts, 50 milliamps, and 30 watts. The settings of the power supply were reduced proportionally with the size of the gel. Each gel was prefocused for 10 minutes, samples were applied, and the gel was run for 1.5 hours (final voltage 1,500 volts). The pH gradient in the gel was measured with a surface electrode.

Staining. After isoelectric focusing, the gels were submerged in buffer (the same buffer used in the stain-mixture) for 3 minutes to remove excess electrolytes that may have been on the surface of the gel. The gels were submerged in a plastic box previously lined with plastic film and filled with the reaction mixtures. A list of the enzymes studied, and references to the stain and reaction mixtures are given in Table 4-1. The gels were incubated in the dark at 25 C until bands appeared. After staining, the gels were photographed and allowed to air dry. The dried gels were covered with a plastic preserving sheet (LKB, Bromma, Sweden) for future reference.

Number of replications. Replications varied with the enzyme system and the populations tested. Data reported here include results obtained after the system was tested

Table 4-1. Enzymes examined, activity, and references to stains and reaction mixtures used in isoelectric focusing of Pratylenchus brachyurus mass homogenates.

Enzyme	E.C.[a] number	Activity[b]	Reference
Oxidoreductases			
Aldehyde oxidase	1.2.3.1	-	89
α-Glycerophosphate dehydrogenase	1.2.1.12	-	7
Isocitrate dehydrogenase	1.1.1.42	+	7
Malate dehydrogenase	1.1.1.37	+	7
Malic enzyme	1.1.1.40	-	72
Octanol dehydrogenase	1.1.1.73	-	72
Phosphogluconate dehydrogenase	1.1.1.44	+	89
Superoxide dismutase	1.15.1.1	-	89
Xanthine dehydrogenase	1.2.1.37	-	72
Transferases			
Glutamate oxaloacetate transaminase	2.6.1.1	-	89
Hexokinase	2.7.1.1	-	72
Phosphoglucomutase	2.7.5.1	+	89
Hydrolases			
Acid phosphatase	3.1.3.2	-	89
Alkaline phosphatase	3.1.3.1	-	89
Esterase	3.1.1.8	-	7
Lyases			
Fumarase	4.2.1.2	-	7
Isomerases			
Manose phosphate isomerase	5.3.1.8	-	72
Phosphoglucose isomerase	5.3.1.9	+	89

[a]Enzyme commission number. Enzyme Committee Union of Biochemistry Classification.
[b]+ = activity detected; - = no activity detected.

thoroughly, and results were obtained at least twice. For example, 11 replicates in seven different runs were done for population 101 in testing the malate dehydrogenase (MDH) system. Populations 101, 102, 103, and 105 were tested together twice with two replications each for the MDH, phosphoglucomutase (PGM), and phosphoglucose isomerase (PGI) systems. Results from population 108 were obtained by comparing it to one of the other four populations.

Paired affinity indices. To compare shared enzymes among the populations, paired affinity (PA) indices (16) were calculated for each population for each of the isozymes used. The PA index was obtained as follows:

$$PA = \frac{\text{Bands in common for pop. A and pop. B}}{\text{Total bands in pop. A + pop. B}}$$

Affinities among the five P. brachyurus populations were visualized by plotting PA indices in polar ordination on the first axis (58). The two populations comprising the most dissimilar population pair (the ones with the lowest PA index) were chosen as the end points of the first axis. The length of the line between the end points is D= 100-PA. All the other paired comparisons were located in relation to the end points. To determine the position of a specific population in relation to the end points, the D value for each of the two end points was calculated. Using the scale

of the axis, a compass was used to mark off an arc of D units from each of the two end points. The intersection of the two arcs determined the position of the population.

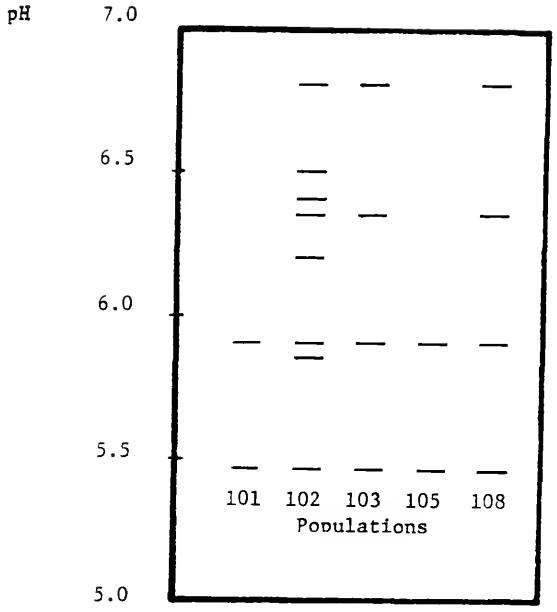
Results

Of the 18 enzyme systems studied, only the MDH, PGM, and PGI systems were resolved efficiently (Figures 4-3, 4-4, 4-5). The isocitrate dehydrogenase (ICD) and phosphogluconate dehydrogenase (PGD) systems showed faint bands. Only MDH, PGM, and PGI were resolved for all five populations, whereas ICD and PGD were resolved for only population 105. No other enzyme system was detected (Table 4-1).

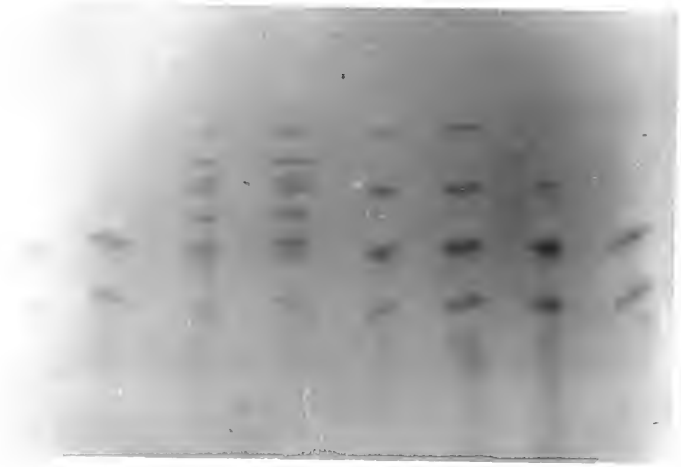
The MDH system contained three distinct phenotypic groups (Figure 4-3). Two electromorphs (electromorph is a single resolved band) were resolved for populations 101 and 105 at pH 5.5 and 5.9. Populations 103 and 108 shared the two electromorphs from group one, but had two others at pH 6.4 and 6.8. Population 102 contained eight electromorphs. Two were shared with all four populations, two others were shared with populations 103 and 108 at pH 6.4 and 6.8. The remaining four electromorphs at pH 5.9, 6.2, 6.4, and 6.5 were unique to population 102.

Three distinct phenotypes were detected in the PGM system (Figure 4-4). Population 101 had three electromorphs located at pH 5.5, 6.1, and 6.7. Populations 103, 105, and

Figure 4-3. Diagrammatic sketch (A) and photograph (B) of patterns of malate dehydrogenase phenotypes following isoelectric focusing of crude protein homogenates from 250 females from each of five Pratylenchus brachyurus populations. Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina; 108- Citrus sp., Polk County, Florida.



(A)

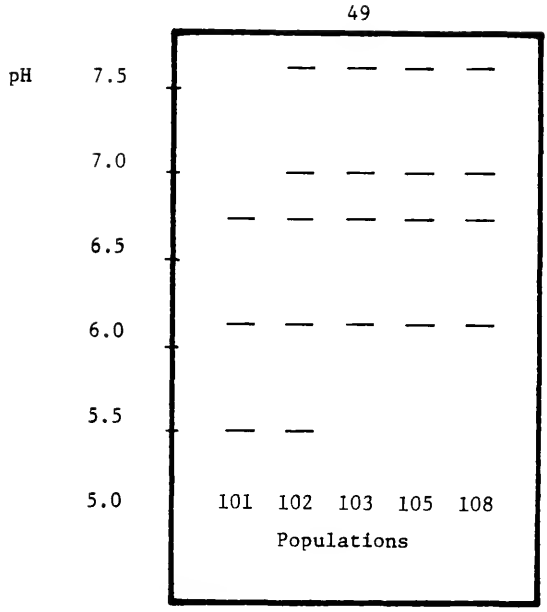


101 101 102 102 103 103 105 105

Populations

(B)

Figure 4-4. Diagrammatic sketch (A) and photograph (B) of patterns of phosphoglucomutase phenotypes following isoelectric focusing of crude protein homogenates from 250 females from each of five Pratylenchus brachyurus populations. Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina; 108- Citrus sp., Polk County, Florida.



(A)

101 101 102 102 103 103 105 105

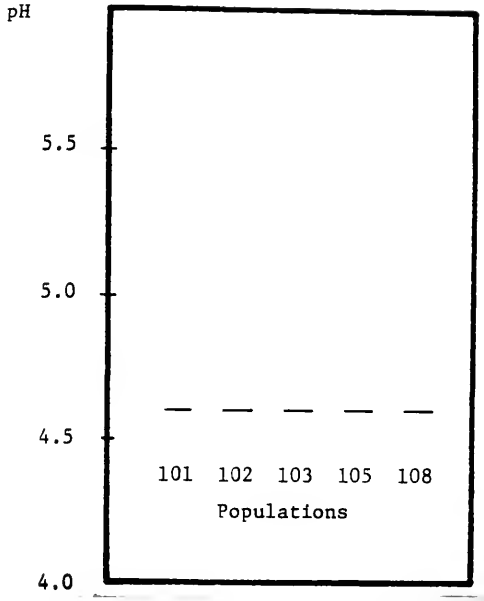
Populations

(B)

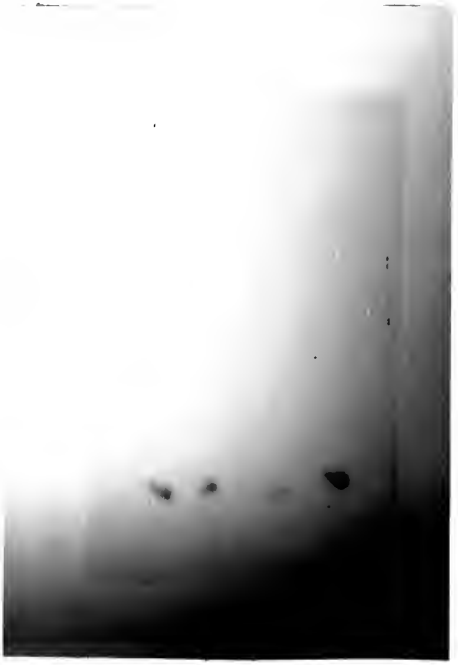
108 shared two electromorphs with populations 101 (pH 6.1 and 6.7), but had two others located at pH 7.0 and 7.6. Population 102 contained five electromorphs. Two were shared with all populations (pH 6.1 and 6.7), one with population 101 (pH 5.5), and two with the other three populations (pH 7.0 and 7.6). The PGI system exhibited only one migrating electromorph at pH 4.6 for all populations (Figure 4-5).

Population 105 showed one faint band at pH 7.0 in the ICD system, and one at pH 5.6 in the PGD system. When comparing females and juveniles of population 105 using the PGI stain system, no phenotypic differences were observed. Phenotypes obtained in the MDH system were the same for nematodes extracted from greenhouse cultures on 'Harvester' snap bean and nematodes from excised root cultures of 'Iochief' corn.

Figure 4-5. Diagrammatic sketch (A) and photograph (B) of patterns of phosphoglucose isomerase phenotypes following isoelectric focusing of crude protein homogenates from 250 females from each of five Pratylenchus brachyurus populations. Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina; 108- Citrus sp., Polk County, Florida.


pH
5.5
5.0
4.5
4.0
101 102 103 105 108
Populations

(A)


101 102 103 105
Populations

(B)

The populations with the most similarity among the enzyme systems tested were 103 from Georgia and 108 from Florida (Table 4-2). Their paired affinity (PA) was 100%. These two P. brachyurus populations had the same phenotypes in all enzyme systems. The populations with the least similarity among the enzyme systems tested were 101 and 102 from Florida with a PA index of 43%. Population 105 from North Carolina was more closely related to 103 and 108 than to 101 or 102 (Figure 4-6).

Discussion

The amount of protein from a single female nematode was insufficient for the detection of enzymes using isoelectric focusing electrophoresis in conjunction with certain enzyme-staining systems. Consequently homogenates were prepared from 250 females. Of the 18 enzyme systems studied, only the MDH, PGM, and PGI enzyme systems were resolved efficiently with 250 females per test. Although the ICD and PGD systems were detected, the bands were faint and more work will be necessary to improve the resolution. Extensive work was done in an attempt to detect esterases, but although excellent resolution was repeatedly obtained with a single female Meloidogyne javanica (Treub, 1885) Chitwood, 1949, no esterase bands were obtained with any Pratylenchus species tested. The lack of resolution of some enzymes in this system may be related to their age because it was learned

Table 4-2: Paired affinity indices of five Pratylenchus brachyurus populations based on three enzyme phenotypes.

Population[a]	101	102	103	105	108
101		6/14[b] 43[c]	5/10 50	5/8 62	5/10 50
102			9/14 64	7/14 50	9/14 64
103				7/9 78	9/9 100
105					7/9 78

Enzymes: malate dehydrogenase, phosphoglucomutase, and phosphoglucose isomerase.

[a]Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County Georgia; 105- 'Forrest' soybean, North Carolina; 108- Citrus sp., Polk County, Florida.

[b]Paired affinity index (PA) is defined as the number of bands in common / total number of bands.

[c]PA%

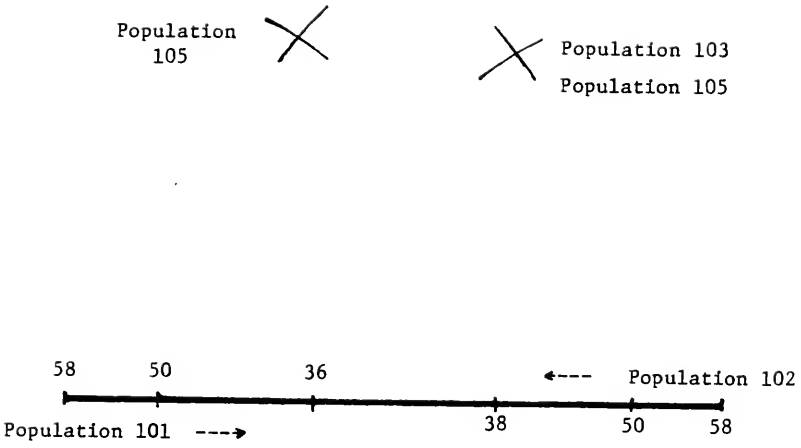


Figure 4-6. Polar ordination of five Pratylenchus brachyurus populations on the first axis. Population origins: 101- 'Pioneer 304C' corn, Alachua County, Florida; 102- 'Florunner' peanut, Alachua County, Florida; 103- 'Florunner' peanut, Tift County, Georgia; 105- 'Forrest' soybean, North Carolina; 108- Citrus sp., Polk County, Florida. Units above the line correspond to population 102, and units below the line correspond to population 101.

that, in at least some cases, nematodes used for protein extraction must be fresh (less than 3 days following extraction from roots) for enzyme bands to be detected. Nematodes stored in water more than 4 days had missing or faint electromorphs (personal observation). Unfortunately, these observations were discovered at a later date and experiments could not be repeated before this report was completed.

Protein extraction from small numbers of vermiform nematodes (less than 250) presents a technique problem. Techniques such as sonication and freezing (liquid nitrogen or -31 C) were attempted without success. It was clear that to detect enzymes from vermiform nematodes, it was necessary to increase the number of nematodes or to reduce the volume of liquid in which they were homogenized. Since the objective of this work was to use the smallest number of nematodes possible, the amount of liquid was reduced to a film of water. Excellent PGI phenotypic resolution was obtained using 100 juveniles (third and fourth stages) from P. brachyurus population 105 from North Carolina. However, for consistency 250 nematodes were used throughout all test.

The MDH and PGM systems indicated distinct levels of genetic variability among populations from various geographical locations. Pratylenchus brachyurus is a monosexual species and reproduces by mitotic parthenogenesis. It has a relatively high chromosome number

(30-32) which also indicates polyploidy (69). To understand the genes involved in the different enzyme systems in plant-parasitic nematodes, it is necessary to design studies with controlled single-pair crosses and identifiable mutant forms that serve as markers. This type of study is difficult with a parthenogenic species such as P. brachyurus. Esterase polymorphism was studied in H. glycines using controlled single-pair crosses (22).

The number of electromorphs detected in the MDH systems during this study is similar to the number observed in other nematodes. From 291 Meloidogyne spp. populations tested using disc-electrophoresis, eight bands of MDH activity were detected (20). One migrating electromorph was characteristic of M. hapla, whereas M. arenaria (Neal, 1889) Chitwood 1949, M. incognita, and M. javanica had similar MDH phenotypes that varied from one to five bands (20). The MDH phenotypes do not differ in females extracted from different hosts (20). Four migrating bands were detected for M. arenaria, and three each for M. hapla, M. incognita, and M. javanica (13). There are three bands for M. incognita females, and four for juvenile stages and eggs (13). Three Florida populations of M. javanica have similar MDH phenotypes with three electromorphs in each population (19). The four-banded MDH phenotype of Meloidogyne incognita separates it from M. arenaria which has only three bands (41). The two races (two species now) of Radopholus similis can be separated

through MDH phenotypes. The banana race (R. similis) has one band and the citrus race (R. citrophilus) has two bands (36).

Several interpretations are possible for the distinct phenotypic groups found in the MDH system in P. brachyurus. First, the multiple bands may represent isozymes, that is, gene products of slightly variant genes. In population 102 there may be eight isozymes involved. Whereas four isozymes may be associated with populations 103 and 108, and two isozymes with populations 101 and 105. It is possible that some of these isozymes were either not present or not expressed in populations with fewer bands. Secondly, since MDH is a diameric enzyme, it is possible that the monomeric units may react enzymatically and break down due to the extraction procedure. Whether or not some of these bands represent artifacts is unknown.

Distinct phenotypic groups were also found in the PGM systems in P. brachyurus. In addition to the interpretations given for MDH systems, it is possible that there were two distinct groups of populations with distinct phenotypes. One group included populations 101, and the other group included populations 103, 105, and 108. Population 102 may be a mixture of the two groups. Thirty populations of Meloidogyne spp. showed seven different PGM phenotypes ranging from four to six bands following starch electrophoresis (21). The two

races (species) of R. similis have identical PGM phenotypes with two bands each (35).

There was only one phenotype for P. brachyurus populations in the PGI system; it could be the product of a single locus. A three-band phenotype was observed for races 3, 4, and 5 of H. glycines, and a one-band phenotype was observed for race 1 after IEF of single females protein (34). Intraspecific variability was detected in 12 H. glycines populations; three different phenotypic groups were obtained (65). Four populations had a single electromorph at pH 4.6, one had none, and seven populations had two electromorphs at pH 4.6 and 4.4. With the use of starch-gel electrophoresis, 10 different phenotypes ranging from one to seven bands were obtained in 30 Meloidogyne spp. populations (21). Also, with starch electrophoresis, six populations of the citrus race and seven populations of the banana race were shown to have two bands. On the other hand, two populations of the banana race had only one band (35).

Isoelectric focusing in conjunction with certain enzyme-staining systems was successful in detecting intraspecific variability within P. brachyurus populations. This technique was sensitive enough to detect polymorphism within populations, as was reported from studies on populations of Heterodera glycines (65), and it provides a useful tool for studying protein polymorphism and genetic diversity among nematode populations (65).

Although more data will be necessary to determine a more conclusive relationship among P. brachyurus populations, it is observed that the two populations with the least affinity were collected from areas that were only 2.4 km apart. Population 103 from peanut in Georgia had the same enzyme phenotypes as population 108 from citrus in Florida.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Morphological studies plus two differentiating techniques (host response and isoelectric focusing electrophoresis) were used to study intraspecific variation among Pratylenchus brachyurus populations selected from different geographical locations and hosts. Different results obtained from the two techniques do not support the hypothesis of similarity among the techniques. There were no extant morphological differences nor behavioral differences, but there were differences in isozyme phenotypes resolved by isoelectric focusing electrophoresis.

The use of host differentials revealed no behavioral differences among the four P. brachyurus populations studied on seven different crop plants. No nematode population affected fresh or root weight when compared to the noninoculated controls. Final nematode population densities showed that none of the populations were different from each other. Although P. brachyurus is pathogenic to citrus (6), none of the populations reproduced in Citrus limon under the conditions of this study.

Isoelectric focusing electrophoresis was investigated to obtain enzyme phenotypes of females from the five P. brachyurus populations. Although there were differences in enzyme phenotypes among the nematode populations in the malate dehydrogenase (MDH) and in the phosphoglucomutase (PGM) systems, the phosphoglucose isomerase (PGI) system showed only one phenotype for all populations. Three distinct phenotypic groups for MDH and PGM systems were resolved. In the MDH system, populations 101 and 105 had two migrating electromorphs. Populations 103 and 108 had two electromorphs from group one plus two others. Population 102, in addition to the two electromorphs shared with all four populations and the two shared with the second group, had four more electromorphs. In the PGM system, population 101 had three electromorphs. Populations 103, 105, and 108 shared two electromorphs with population 101, but had two others. Population 102 had all five electromorphs. Two were shared with all populations, one with population 101, and two with the other three populations. The PGI system showed only one migrating electromorph for all populations.

Based on enzyme phenotypes, population 103 from North Carolina and population 108 from Florida were similar because both populations had the same enzyme phenotype for all three enzyme systems. On the other hand, populations 101 and 102 from Florida were the least similar even though they were collected only 2.4 km apart. This study showed that

isoelectric focusing in conjunction with enzyme-specific stain systems provided a good tool to detect intraspecific variations within P. brachyurus populations. More enzyme systems must be studied before a more conclusive relationship can be determined among P. brachyurus populations. Also, the role of enzyme systems is unclear and warrants further studies.

APPENDIX
FINAL POPULATION DENSITIES OF FOUR PRATYLENCHUS BRACHYURUS POPULATIONS ON SELECTED HOSTS 64 DAYS AFTER INOCULATION

Table A-1. Final population densities (nematodes per 10 g of root) of Pratylenchus brachyurus originating from 'Pioneer 304C' corn, Alachua County, Florida, on selected plants 64 days after inoculation.

Plant species	Year	Replicate					
		I	II	III	IV	V	VI
Alfalfa	1986	344	130	70	22	676	26
	1987	3	11	3	8	4	
Snap bean	1986	1,888	2,149	444	883	3,742	239
	1987	157	99	157	537	141	
Citrus	1986	0	4	1	0	0	2
	1987	9	5	5	8	7	
Corn	1987	871	186	190	13	385	90
	1986	106	61	272	148	29	
Peanut	1986	36	77	129	79	41	10
	1987	41	93	64	70	48	
Soybean	1986	169	523	118	7	458	131
	1987	203	667	93	123	70	
Tomato	1986	772	1337	777	716	1,344	251
	1987	60	384	37	25	9	

Table A-2. Final population densities (nematodes per 10 g of root) of Pratylenchus brachyurus originating from 'Florunner' peanut, Alachua County, Florida, on selected plants 64 days after inoculation.

Plant species	Year	Replicate					
		I	II	III	IV	V	VI
Alfalfa	1986	120	684	1268	78	255	469
	1987	4	35	0	92	3	
Snap bean	1986	1,831	5,229	293	4,127	--[a]	1,178
	1987	78	105	124	63	87	
Citrus	1986	0	0	0	0	0	0
	1987	7	7	5	5	6	
Corn	1987	537	247	85	347	230	76
	1986	53	96	34	54	8	
Peanut	1986	24	105	225	91	57	24
	1987	26	57	56	42	21	
Soybean	1986	221	531	149	128	90	0
	1987	38	193	40	85	26	
Tomato	1986	328	322	491	274	270	785
	1987	31	42	4	25	3	

[a]Missing value.

Table A-3. Final population densities (nematodes per 10 g of root) of Pratylenchus brachyurus originating from 'Florunner' peanut, Tift County, Georgia, on selected plants 64 days after inoculation.

Plant species	Year	Replicate I	II	III	IV	V	VI
Alfalfa	1986	628	150	153	343	41	410
	1987	1	9	10	25	1	
Snap bean	1986	3,663	3,564	3,936	2,551	1,323	904
	1987	83	186	132	50	14	
Citrus	1986	0	0	1	3	0	3
	1987	2	3	2	2	1	
Corn	1987	503	431	102	313	177	258
	1986	29	26	121	11	8	
Peanut	1986	174	252	213	58	151	100
	1987	38	24	125	37	19	
Soybean	1986	266	171	576	152	183	479
	1987	83	98	81	71	117	
Tomato	1986	668	371	419	114	493	602
	1987	25	36	14	21	16	

Table A-4. Final population densities (nematodes per 10 g of root) of Pratylenchus brachyurus originating from 'Forrest' soybean, North Carolina, on selected plants 64 days after inoculation.

Plant species	Year	Replicate					
		I	II	III	IV	V	VI
Alfalfa	1986	94	428	96	64	70	82
	1987	4	5	2	6	2	
Snap bean	1986	1,313	747	199	2,632	1,837	744
	1987	25	16	21	50	73	
Citrus	1986	0	0	2	1	0	0
	1987	1	2	2	0	0	
Corn	1987	40	295	297	120	148	156
	1986	42	27	32	106	9	
Peanut	1986	136	41	70	83	44	20
	1987	21	46	23	24	10	
Soybean	1986	114	149	31	296	84	0
	1987	73	86	100	63	27	
Tomato	1986	115	378	797	211	421	250
	1987	7	21	16	25	5	

LITERATURE CITED

1. Andrews, A. T. 1981. Electrophoresis: Theory, techniques, and biochemical and clinical applications. Oxford: Clarendon Press.

2. Bajaj, H. K., and D. S. Bhatti. 1984. New and known species of Pratylenchus Filipjev, 1936 (Nematoda: Pratylenchidae) from Haryana, India, with remarks on intraspecific variations. Journal of Nematology 16:360-367.

3. Baldwin, J. G., and T. O. Powers. 1987. Use of fine structure and nucleic acid analysis in systematics. Pp. 336-345 in J. A. Veech and D. W. Dickson, eds. Vistas in Nematology: A commemoration of the twenty-fifth anniversary of the Society of Nematologists. Hyattsville, Maryland: Society of Nematologists.

4. Bolla, R. I., C. Weaver, P. Koslowski, K. Fitzsimmoms, and R. E. K. Winter. 1987. Characterization of a nonparasitic isolate of Bursaphelenchus xylophilus. Journal of Nematology 19:304-310.

5. Bolla, R. I., C. Weaver, and R. E. K. Winter. 1988. Genomic differences among pathotypes of Bursaphelenchus xylophilus. Journal of Nematology 20:309-316.

6. Brooks, T. L., and V. G. Perry. 1967. Pathogenicity of Pratylenchus brachyurus to citrus. Plant Disease Reporter 51:569-573.

7. Bush, G. L., and R. N. Huettel. 1972. Starch gel electrophoresis of tephrid proteins: A manual of techniques. International Biological Programme, Working Group on Fruit Flies. Population Genetics Project, Phase I. New York: Academic Press. 56 pp.

8. Chow, H. H., and J. Pasternak. 1969. Protein changes during maturation of the freeliving nematode, Panagrellus silusiae. Journal of Experimental Zoology 170:77-84.

9. Corbett, D. C. M. 1969. Pratylenchus pinguicaudatus n. sp. (Pratylenchinae: Nematoda) with a key to the genus Pratylenchus. Nematologica 15:550-556.

10. Corbett, D. C. M. 1976. Pratylenchus brachyurus. Descriptions of plant-parasitic nematodes. Set No. 6. London: Commonwealth Institute of Helminthology.

11. Corbett, D. C. M., and S. A. Clark. 1983. Surface features in the taxonomy of Pratylenchus species. Revue de Nematologie 6:85-98.

12. Curran, J., M. A. McClure, and J. M. Webster. 1986. Genotypic differentiation of Meloidogyne populations by detection of restriction fragment length difference in total DNA. Journal of Nematology 18:83-86.

13. Dickson, D. W., D. Huisingh, and J. N. Sasser. 1971. Dehydrogenases, acid and alkaline phosphatases, and esterases for chemotaxonomy of selected Meloidogyne, Ditylenchus, Heterodera and Aphelenchus spp. Journal of Nematology 3:1-16.

14. Dickson, D. W., J. N. Sasser, and D. Huisingh. 1970. Comparative disc-electrophoretic protein analysis of selected Meloidogyne, Ditylenchus, Heterodera, and Aphelenchus spp. Journal of Nematology 2:286-293.

15. Dropkin, V. H. 1988. The concept of race in phytonematology. Annual Review of Phytopathology 26:145-161.

16. Ellison, W. L., R. E. Alston, and B. L. Turner. 1962. Methods of presentation of crude biochemical data for systematic purposes, with particular reference to the genus Bahia (Compositae). American Journal of Botany 49: 599-604.

17. Endo, B. Y. 1959. Responses of root-lesion nematodes, Pratylenchus brachyurus and P. zeae, to various plants and soil types. Phytopathology 49:417-421.

18. Endo, B. Y. 1967. Comparative population increase of Pratylenchus brachyurus and P. zeae in corn and soybean varieties Lee and Peking. Phytopathology 57:118-120.

19. Erba, P. S., and D. W. Dickson. 1979. Disc electrophoretic analysis of two Florida populations of Meloidogyne javanica. Journal of Nematology 11:297 (Abstr.).

20. Esbenshade, P. R., and A. C. Triantaphyllou. 1985. Use of enzyme phenotypes for identification of Meloidogyne species. Journal of Nematology 17:6-20.

21. Esbenshade, P. R., and A. C. Triantaphyllou. 1987. Enzymatic relationships and evolution in the genus Meloidogyne (Nematoda: Tylenchida). Journal of Nematology 19:8-18.

22. Esbenshade, P. R., and A. C. Triantaphyllou. 1988. Genetic analysis of esterase polymorphism in the soybean cyst nematode, Heterodera glycines. Journal of Nematology 20:486-492.

23. Ferris, V. R., J. N. Ferris, and L. L. Murdock. 1987. Two-dimensional protein patterns in Labronema, Aporcelaimellus, and Eudorylaimus (Nematoda: Dorylaimida). Journal of Nematology 19:431-440.

24. Filipjev, I. N. 1934. The classification of the free-living nematodes and their relation to the parasitic nematodes. Smithsonian Miscellaneous Collection. Publication 3216, Leiden: E. J. Brill. 66 pp.

25. Filipjev, I. N. 1936. On the classification of the Tylenchinae. Proceedings of the Helminthological Society of Washington 3:80-82.

26. Ford, H. W. 1957. A source of controlled vacuum for pipetting nematodes. Plant Disease Reporter 14:89-90.

27. Frederick J. J., and A. C. Tarjan. 1978. Variability in measurements made of same nematode specimen by various observers or by one observer on different days. Nematologica 24:476-478.

28. Frederick J. J., and A. C. Tarjan. In press. A compendium of the genus Pratylenchus Filipjev, 1936. (Nemata: Pratylenchidae). Revue de Nematologie.

29. Godfrey, G. H. 1929. A destructive root disease of pineapples and other plants due to Tylenchus brachyurus n. sp. Phytopathology 19:611-629.

30. Good, J. M., L. W. Boyle, and R. O. Hammons. 1958. Studies of Pratylenchus brachyurus on peanuts. Phytopathology 48:530-535.

31. Hartman, K. M., and J. N. Sasser. 1985. Identification of Meloidogyne species on the basis of differential host test and perineal-pattern morphology. Pp. 69-77 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on Meloidogyne, Vol. II. Methodology. Raleigh: North Carolina State University Graphics.

32. Heald, C. M. 1987. Classical nematode management practices. Pp. 100-104 in J. A. Veech and D. W. Dickson, eds. Vistas in Nematology: A commemoration of the twenty-fifth anniversary of the Society of Nematologists. Hyattsville, Maryland: Society of Nematologists.

33. Huettel, R. N. 1982. Genetic bases for identification and separation of the two Florida races of Radopholus similis (Cobb) Thorne. Ph. D. dissertation. Gainesville: University of Florida.

34. Huettel, R. N. 1986. Analysis of phosphoglucose isomerase from single female soybean cyst nematodes, races 1, 3, 4, and 5 by isoelectric focusing. Journal of Nematology 18:613 (Abstr.).

35. Huettel, R.N., D. W. Dickson, and D. T. Kaplan. 1983. Biochemical identification of the two races of Radopholus similis by starch gel electrophoresis. Journal of Nematology 15:338-344.

36. Huettel, R.N., D. W. Dickson, and D. T. Kaplan. 1983. Biochemical identification of the two races of Radopholus similis by polyacrylamide gel electrophoresis. Journal of Nematology 15:345-348.

37. Huettel, R. N., and R. V. Rebois. 1985. Culturing plant parasitic nematodes using root explants. Pp. 155-158 in B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant nematology, laboratory manual. Amherst: University of Massachusetts Agricultural Experiment Station.

38. Hussey, R. S. 1972. Serological relationship of Meloidogyne incognita and M. arenaria. Journal of Nematology 4:101-104.

39. Hussey, R. S. 1979. Biochemical systematics of nematodes--a review. Commonwealth Institute of Helminthology, Helminthological Abstracts. Series B, Plant Nematology 48:141-148.

40. Hussey, R. S., and L. R. Krusberg. 1971. Disc-electrophoresis patterns of enzymes and soluble proteins of Ditylenchus dipsaci and D. triformis. Journal of Nematology 3:79-84.

41. Hussey, R. S., J. N. Sasser, and D. Huisingh. 1972. Disc-electrophoretic studies of soluble proteins and enzymes of Meloidogyne incognita and M. arenaria. Journal of Nematology 4:183-189.

42. Hyman, B. C. 1988. Nematode mitochondrial DNA: anomalies and applications. Journal of Nematology 20:523-531.

43. Ishibashi, N. 1970. Variations of the electrophoretic protein patterns of Heteroderidae (Nematoda: Tylenchida) depending on the developmental stages of the nematode and on the growing conditions of the host plant. Applied Entomology and Zoology 5:23-32.

44. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

45. Johnson, A. W. 1985. Specific crop rotation effects combined with cultural practices and nematicides. Pp. 238-301 in J. N. Sasser and C. C. Carter, eds. An advanced treatise on Meloidogyne, Vol. I. Biology and control. Raleigh: North Carolina State University Graphics.

46. Johnson A. W., and J. Feldmesser. 1987. Nematicides--a historical review. Pp. 448-454 in J. A. Veech and D. W. Dickson, eds. Vistas in Nematology: A commemoration of the twenty-fifth anniversary of the Society of Nematologists. Hyattsville, Maryland: Society of Nematologists.

47. Kalinski, A., and R. N. Huettel. 1988. DNA restriction fragment length polymorphism in races of the soybean cyst nematode, Heterodera glycines. Journal of Nematology 20:532-538.

48. Koen, H. 1967. Notes on the host range, ecology and population dynamics of Pratylenchus brachyurus. Nematologica 13:118-124.

49. Krusberg, L. R., R. S. Hussey, and C. L. Fletcher. 1973. Lipid and fatty acid composition of females and eggs of Meloidogyne incognita and M. arenaria. Comparative Biochemistry and Physiology 45B:335-341.

50. Lawson, E. C. III, G. E. Carter, Jr., and S. A. Lewis. 1984. Application of isoelectric focusing to the taxonomic identification of Meloidogyne spp. Journal of Nematology 16:91-96.

51. Lindsey, D. W., and E. J. Cairns. 1971. Pathogenicity of the lesion nematode, Pratylenchus brachyurus, on six soybean cultivars. Journal of Nematology 3:220-226.

52. Loof, P. A. A. 1960. Taxonomic studies on the genus Pratylenchus (Nematoda). Tijdschr Plantenziekten 66:29-90.

53. Loof, P. A. A. 1978. The genus Pratylenchus Filipjev, 1936 (Nematoda: Pratylenchidae): A review of its anatomy, morphology, distribution, systematics and identification. Uppsala: Swedish University of Agricultural Sciences, 50 pp.

54. Misaghi, I., and M. A. McClure. 1974. Antigenic relationship of Meloidogyne incognita, M. javanica, and M. arenaria. Phytopathology 64:698-701.

55. O'Bannon, J. H., and A. T. Tomberlin. 1970. Pratylenchus spp. as citrus pathogens. Phytopathology 60:1540 (Abstr.).

56. Olowe, T., and D. C. M. Corbett. 1984. Morphology and morphometrics of Pratylenchus brachyurus and P. zeae II. Influence of environmental factors. Indian Journal of Nematology 14:6-17.

57. Olowe, T., and D. C. M. Corbett. 1984. Morphology and morphometrics of Pratylenchus brachyurus and P. zeae III. Influence of geographical location. Indian Journal of Nematology 14:30-35.

58. Poole, R. W. 1974. An introduction to quantitative ecology. New York: McGraw-Hill Book Company. 532 pp.

59. Powers, T. O., E. G. Platzer, B. C. Hyman. 1986. Species-specific restriction site polymorphism in root-knot nematode mitochondrial DNA. Journal of Nematology 18:288-293.

60. Powers, T. O., and L. J. Sandall. 1988. Estimation of genetic divergence in Meloidogyne mitochondrial DNA. Journal of Nematology 20:505-511.

61. Pozdol, R. F., and G. R. Noel. 1984. Comparative electrophoretic analysis of soluble proteins from Heterodera glycines races 1-4 and three other Heterodera species. Journal of Nematology 16:332-340.

62. Radewald, J. D., J. H. O'Bannon, and A. T. Tomberlin. 1971. Temperature effects on reproduction and pathogenicity of Pratylenchus coffeae and P. brachyurus and survival of P. coffeae in roots of Citrus jambhiri. Journal of Nematology 3:390-394.

63. Radice, A. D., T. O. Powers, L. J. Sandall, and R. D. Riggs. 1988. Comparisons of mitochondrial DNA from the sibling species Heterodera glycines and H. schachtii. Journal of Nematology 20:443-450.

64. Radice, A. D., R. D. Riggs, and F. H. Huang. 1985. Protein and enzyme polymorphism of Heterodera glycines. Journal of Nematology 17:510 (Abstr.).

65. Radice, A. D., R. D. Riggs, and F. H. Huang. 1988. Detection of intraspecific diversity of Heterodera glycines using isozyme phenotypes. Journal of Nematology 20:29-39.

66. Riggs, R. D., M. L. Hamblen, and L. Rakes. 1981. Infra-species variation in reactions to hosts in Heterodera glycines populations. Journal of Nematology 13:171-179.

67. Righetti, P. G. 1983. Isoelectric focusing: Theory, methodology and applications. Pp. 1-135 in T. S. Work and R. H. Burdon, eds. Laboratory techniques in biochemistry and molecular biology. Amsterdam: Elsevier Biomedical Press.

68. Roman, J., and H. Hirschmann. 1969. Morphology and morphometrics of six species of Pratylenchus. Journal of Nematology 1:363-386.

69. Roman, J., and A. C. Triantaphyllou. 1969. Gamtogenesis and reproduction of seven species of Pratylenchus. Journal of Nematology 1:357-362.

70. Sasser, J. N., and D. W. Freckman. 1987. A world perspective in nematology: The role of the Society. Pp. 7-14 in J. A. Veech and D. W. Dickson, eds. Vistas in Nematology: A commemoration of the twenty-fifth anniversary of the Society of Nematologists. Hyattsville, Maryland: Society of Nematologists.

71. Schmitt, D. P., and K. R. Barker. 1981. Damage and reproductive potentials of Pratylenchus brachyurus and P. penetrans on soybean. Journal of Nematology 13:327-332.

72. Shaw, C., and R. Prasad. 1970. Starch gel electrophoresis of enzymes--a compilation of recipes. Biochemical Genetics 4:297-320.

73. Sher, S. A., and M. W. Allen. 1953. Revision of the genus Pratylenchus (Nematoda: Tylenchidae) University of California Publications in Zoology 57:441-469.

74. Sidhu, G. S., and J. M. Webster. 1981. The genetics of plant-nematode parasitic systems. The Botanical Review 47:387-419.

75. Singh, D. B., and E. Khan. 1981. Morphological variations in populations of Pratylenchus thornei Sher & Allen, 1953. Indian Journal of Nematology 11:53-60.

76. Southards, C. J., and C. J. Nusbaum. 1967. Genetic variability of tobacco response to Pratylenchus brachyurus. Phytopathology 57:18-21.

77. Southern Regional Technical Committee, S-19. 1960. Distribution of plant-parasitic nematodes in the south. Southern Cooperative Series. Bulletin 74. Washington: United States Department of Agriculture. 72 pp.

78. Southey, J. F. 1978. Plant nematology. London: Her Majesty's Stationery Office, 17 p.

79. Starr, J. L. 1984. Expression of resistance in peanuts to Pratylenchus brachyurus: Impact on screening for resistance. Journal of Nematology 16:404-406.

80. Stoll, N. R., R. Ph. Dollfus, J. Forest, N. D. Riley, C. W. Sabrosky, C. W. Wright, and R. V. Melville, eds. 1964. International code of zoological nomenclature. London: The International Trust for Zoological Nomenclature.

81. Sturhan, D. 1972. Biological races. Pp. 51-71 in B. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes. Vol. 2. New York: Academic Press.

82. Tarjan, A. C., and J. J. Frederick. 1978. Intraspecific morphological variation among populations of Pratylenchus brachyurus and P. coffeae. Journal of Nematology 10:152-160.

83. Tarjan, A. C., and J. H. O'Bannon. 1969. Observations on meadow nematodes (Pratylenchus spp.) and their relation to declines of citrus in Florida. Plant Disease Reporter 53:683-686.

84. Tarte, R., and W. F. Mai. 1976. Morphological variation in Pratylenchus penetrans. Journal of Nematology 8:185-195.

85. Taylor, A. L., and J. N. Sasser. 1978. Biology, identification, and control of root-knot nematodes Meloidogyne species). International Meloidogyne Project. Raleigh: North Carolina State University Graphics, 111 pp.

86. Taylor, D. P., and W. R. Jenkins. 1957. Variation within the nematode genus Pratylenchus, with the descriptions of P. hexincisus, n. sp. and P. subpenetrans, n. sp. Nematologica 2:159-174.

87. Thames, W. H. 1982. The genus Pratylenchus. Pp. 108-126 in R. D. Riggs, ed. Nematology in the southern region of the United States. Southern Cooperative Series Bulletin 276. Fayetteville: University of Arkansas, 206 pp.

88. Trudgill, D. L., and D. M. Parrott. 1972. Disc electrophoresis and larval dimensions of British, Dutch and other populations of Heterodera rostochiensis, as evidence of the existence of two species, each with pathotypes. Nematologica 18:141-148.

89. Vallejos, C. E. 1983. Enzyme activity staining. Pp. 469-516 in S. D. Tanksley, and T. J. Orton, eds. Isozymes in plant genetics and breeding. Part A. Amsterdam: Elsevier Biomedical Press.

BIOGRAPHICAL SKETCH

Luis A. Payan was born on March 26, 1957, in Cali, Colombia. He attended San Luis Gonzaga High School in Cali, Colombia, and graduated in July 1974. After graduation, he attended the University of Colombia, Palmira, Colombia. In 1978 he attended Abraham Baldwin Agricultural College, Tifton, Georgia, on a tennis scholarship. In January 1980 he enrolled at the University of Georgia, Athens, Georgia, where he earned a B.S. degree in agronomy with a double major in pest management. Before enrolling in graduate school, he played professional tennis tournaments in Eastern Europe during 1981. In August 1982 he began graduate studies in the Department of Plant Pathology where he received a M.S. degree from the University of Georgia in March 1985. His research was conducted at the Coastal Plain Experiment Station, Tifton, Georgia, under the direction of Dr. A. W. Johnson and Dr. R. H. Littrell. His thesis was entitled "Effect of nematicides and herbicides alone and in combination on hatching, penetration, development and reproduction of Meloidogyne incognita." In January 1985 he moved to Gainesville, Florida, to conduct research for this dissertation under the direction of Dr. D. W. Dickson.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Mitchell
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the graduate faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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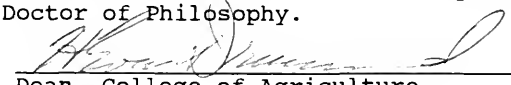

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Dean, Graduate School